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Human Herpesvirus-8 (HHV-8) Associated With Small Non-Cleaved Cell Lymphoma in a Child With AIDS

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The association of human herpesvirus-8 (HHV-8) with a small non-cleaved cell lymphoma is described in a child with the acquired immunodeficiency syndrome (AIDS) who developed a malignant pleural effusion and radiologic evidence of multiple solid tumors. HHV-8 DNA and Epstein-Barr virus DNA were identified in pleural fluid cells by polymerase chain reaction (PCR) amplification. The serum antibody titer against lytic HHV-8 proteins was 1:640; antibodies to latent HHV-8 proteins were not detected. Cytogenetic analysis of malignant cells revealed three abnormal karyotypes sharing the common finding of a t(8;14) translocation. Rearrangement of c-*myc* was demonstrated by PCR analysis. Oligoclonal JH immunoglobulin bands were found. Insufficient pleural fluid cells were available to permit localization of HHV-8 to malignant cells by in situ hybridization. This malignancy contrasts with HHV-8-associated lymphomas reported in adult patients with AIDS with respect to cell morphology, c-*myc* translocation, and oligoclonal immunoglobulin gene rearrangement. HHV-8 is associated with a wider spectrum of malignancies than recognized previously. Am. J. Hematol. 60:215–221, 1999.

Key words: AIDS, small non-cleaved cell lymphoma; human herpesvirus 8

INTRODUCTION

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma (KS)-associated herpesvirus, is a newly-identified human herpesvirus first discovered in KS tissues from adult patients with acquired immunodeficiency syndrome (AIDS) [1]. HHV-8 has been demonstrated in patients with AIDS but without KS [2]; in more than 95% of classic, endemic, and transplant-associated KS in human immunodeficiency virus (HIV)-uninfected individuals [3,4]; multicentric Castleman's disease [5]; and primary effusion lymphoma [6]. Primary effusion lymphoma, also known as body cavity-based lymphoma, is a rare form of non-Hodgkin's lymphoma developing predominantly in patients with AIDS that is characterized by the presence of a malignant effusion in one or more body cavities, generally in the absence of a primary tumor mass [7,8]. All HHV-8-associated primary effusion lym-

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phomas reported have displayed a pleomorphic or immunoblastic large cell morphology.

As a component of the Pediatric AIDS Malignancy Network, a national network for studying malignancies in HIV-infected children, we have examined five malignant effusions from four patients for the presence of HHV-8. HHV-8 DNA was identified in pleural effusion cells from one child with a malignant pleural effusion and multiple solid tumors confirmed by radiologic examinations. Unlike previously-reported lymphomas associated with HHV-8, this child's tumor was a small non-cleaved cell lymphoma.

CASE REPORT

A 4-year-old black female child of a woman with a history of illicit injectable drug use was diagnosed with HIV infection at age 5 months and classified as P_2, A, C, $D_{1,3}$ [9]. Significant medical illnesses included: cytomegalovirus pneumonitis (5 months of age); lymphadenopathy, hepatosplenomegaly, and recurrent thrush (1 year of age); chronic parotitis with intermittently elevated serum lipase (2 years of age); and lymphocytic interstitial pneumonitis (2.5 years of age). *Pneumocystis carinii* prophylaxis with trimethoprim/sulfamethoxazole was initiated at 1 year of age. Antiretroviral therapy included zidovudine beginning at 1 year of age, which was changed to didanonsine beginning at 3 years of age.

At 4.5 years of age, the patient was evaluated for progressive respiratory distress. Physical examination findings included a respiratory rate of 50/min, heart rate 140/min, and cervical, axillary, and inguinal lymphadenopathy as well as hepatosplenomegaly. Laboratory results included: normal serum electrolytes, BUN, and creatinine; AST, 81 U/L; ALT, 20 U/L; LDH, 1,656 U/L; alkaline phosphatase, 3,852 U/L (isoenzyme analysis indicated that the alkaline phosphatase was non-hepatic in origin); amylase, 254 U/L; and lipase, 2,900 U/L. The white blood cell count was 7.6×10^9/L; hemoglobin, 11.0 g/dL; hematocrit, 33%; and platelet count, 387×10^9/L. The peripheral CD4 cell count was 130/μL with a CD4/CD8 ratio of 0.2.

A chest X-ray revealed bilateral pleural effusions and an enlarged cardiac silhouette. Echocardiography showed a moderate pericardial effusion and a 4 cm right atrial mass attached to the tricuspid valve.

Abdominal CT imaging of the abdomen demonstrated a 5.3 × 4.5 × 4.9 cm mass of homogeneous attenuation encircling the body of the stomach impinging upon the left lobe of the liver, filling the lesser sac, and displacing the spleen posteriorly (Figs. 1 and 2). A smaller mass with less enhancement was present in the dome of the liver. Multiple round masses suggestive of focal masses or complex cysts were seen within the kidneys bilaterally. There was extensive retroperitoneal and mesenteric lymphadenopathy. The chest CT revealed large bilateral pleural effusions with uniform fluid density and a small pericardial effusion of intermediate attenuation (Fig. 3). A right atrial mass, extending from the anterolateral wall, was identical in attenuation to the other masses. Nodular masses, consistent with enlarged lymph nodes, were seen in the mediastinum. The posterior portions of the lungs

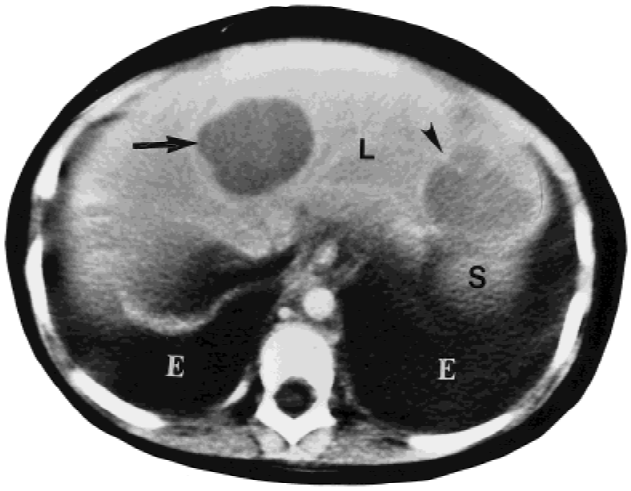

Fig. 1. CT scan of abdomen demonstrating a well-defined mass within the dome of the liver (arrow) with diminished enhancement compared with surrounding liver parenchyma. A second lesion (arrowhead) is seen abutting the lateral segment of the left hepatic lobe and the tip of the spleen. There are large bilateral pleural effusions of fluid density. (L, liver; S, spleen; E, pleural effusion.)

Fig. 2. Contrast-enhanced abdominal CT scan caudad to Figure 1 demonstrating an eccentric mass (curved arrows) encircling the body of the stomach and extending into the lesser sac with displacement of the gastric antrum ventrally. This mass is contiguous with the pancreas and extensive soft tissue which infiltrates the mesentery and retroperitoneum.

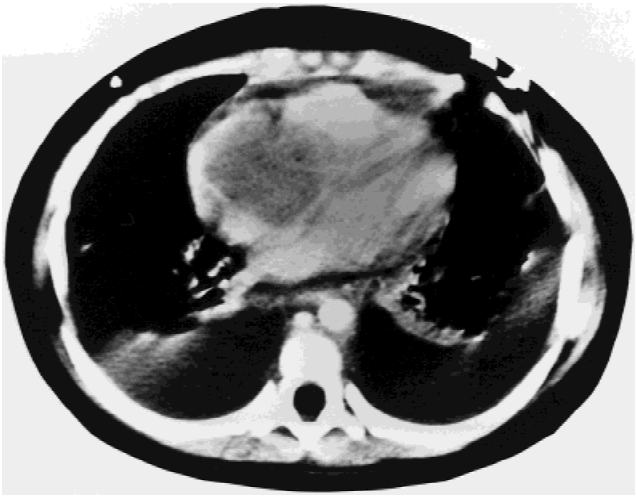

Fig. 3. Axial image through the cardiac chambers demonstrates a large homogeneous mass within the right atrium.

were atelectatic and surrounded by pleural fluid. No intrapulmonary nodules were seen.

Thoracentesis revealed hazy yellow fluid with: RBCs, 20/μL; WBCs, 7,650/μL; protein, 3.3 g/dL; glucose, 87 mg/dL; LDH, 723 U/L; and amylase, 127 U/L. Pathological examination of the pleural fluid cells revealed small non-cleaved lymphoma cells. No other tissues were collected for pathologic examination.

The family refused further invasive diagnostic or staging procedures and declined chemotherapy. Supportive care was provided. The patient developed oliguric renal failure and died 5 days after pleurocentesis. Permission for autopsy was not granted.

MATERIALS AND METHODS

Informed Consent

Informed consent was obtained from the child's mother for the study of biologic specimens according to institutional guidelines.

Pathology

Cytospin preparations of pleural fluid cells were fixed and stained with Papanicolaou stain, and a cell block was prepared, sectioned, and stained with hematoxylin and eosin.

Polymerase Chain Reaction (PCR) Amplification

PCR amplification was performed with 0.67 μg of cellular DNA (peripheral blood mononuclear cells [PBMCs] and pleural fluid cells) or 5 μL of plasma DNA using methods described previously [10,11]. All samples were tested by non-nested PCR [1] and nested PCR [12], each in duplicate, for the presence of HHV-8 DNA sequences using primers from open reading frame (ORF) 26 (capsid protein VP-23). PBMCs and pleural fluid cells were also amplified by non-nested PCR in a different laboratory by a second investigator (SJG) using a primer set from a different region of the genome (set no. 2, outer) [13] from ORF 25 [14]. Epstein-Barr virus (EBV) DNA was tested using an established quantitative PCR method [10].

Serology

Serum antibodies to HHV-8 were assayed by: indirect immunofluorescence assay (IFA) to lytic proteins [15]; IFA to latent proteins [16]; Western blot to latent nuclear antigen [17]; and Western blot to recombinant ORF 65 (lytic protein) [18]. Plasma from an adult with KS was used as a positive control (titer 1:1280), and plasma from healthy HIV-uninfected adult volunteers was used as negative controls.

Cytogenetics

Following short term (24 hr) culture, G-banded chromosomal preparations were obtained from pleural fluid cells utilizing routine procedures.

Immunoglobulin Studies

Immunoglobulin gene clonality was determined for pleural fluid cells by amplifying the Fr3A-JH regions of the immunoglobulin heavy chain gene using oligonucleotide primers described previously [19].

c-*myc* Rearrangements

Rearrangements of c-*myc* were identified by PCR [20] and direct DNA sequencing of PCR products [21].

RESULTS

Pathology

Both the cell block (Fig. 4) and cytospin preparations showed small non-cleaved cell lymphoma. Material was reviewed twice in a blinded manner by two pathologists (DRH and C. Berard), once separately and once together, with identical diagnoses.

PCR Amplification

Pleural fluid cells were positive for HHV-8 DNA by non-nested and also nested PCR of the ORF 26 region (Fig. 5), and by PCR using non-overlapping primers of ORF 25 (data not shown). The controls showed the expected results. PBMCs were weakly positive by nested PCR in ORF 26 (Figure 5B); PBMCs and plasma were negative by non-nested PCR. EBV DNA sequences were present in PBMCs and pleural fluid cells (600 and 9,500 viral genomes per 100,000 cells, respectively) (data not shown).

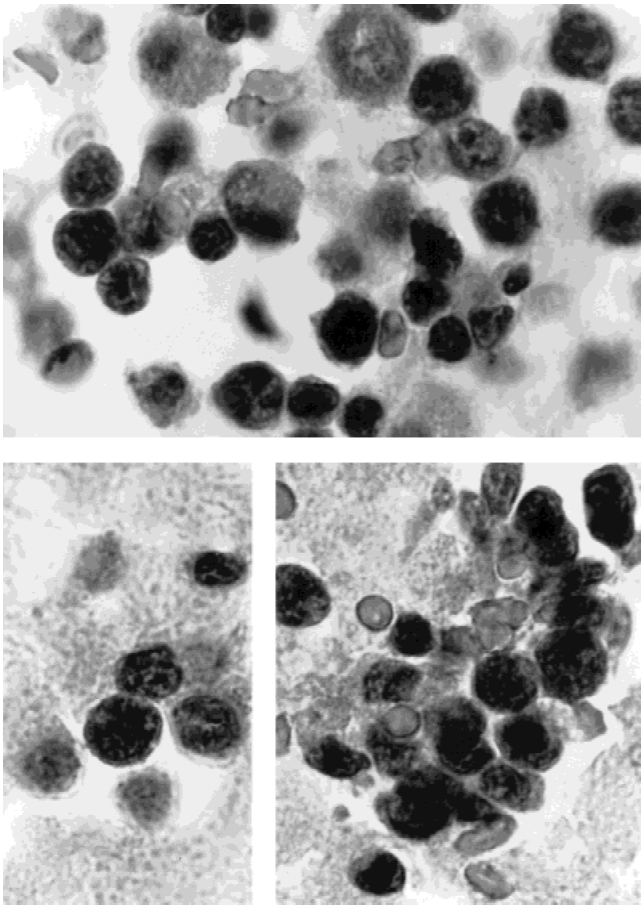

Fig. 4. Cell block preparation of pleural fluid, sectioned and stained with hematoxylin and eosin. An infiltrate is present in cells of intermediate size (15–18 μm in diameter), with slightly coarse chromatin, one to several small nucleoli, and scant-to-moderate amounts of basophilic cytoplasm, typical of small non-cleaved cell lymphoma with this preparatory method. (Magnification, ×250)

Serology

Antibodies to lytic HHV-8 proteins were detected at a titer of 1:640 using IFA. Lytic antibodies to ORF 65 were negative by Western blot. Antibodies to latent proteins were negative.

Cytogenetics

A total of 18 metaphase spreads were analyzed in detail. The pleural fluid cells demonstrated a modal number of 46 chromosomes with complex mosaicism with evidence of three different karyotypes. Multiple translocations were apparent with a common finding of a t(8;14)(q24;q32) translocation. Two different, submetacentric unidentifiable marker chromosomes and a duplication of the 1q31→qter region were noted in some cells. The detailed karyotype was: 46,XX,dup(1)(q31→qter), t(8;14)(q24;q32)[3 cells]/46,XX,t(1;3)(q23;p25),t(8;14)(q24;q32)[8 cells]/46,XX,t(1;3)(q23;p25), t(8;14)(q24;q32),der(8),−10,+mar1,+mar2[7 cells].

Immunoglobulin Studies

Three discrete oligoclonal JH immunoglobulin bands were visualized on ethidium bromide-stained agarose gels.

c-*myc* Rearrangements

Rearrangement of c-*myc* was detected in pleural fluid cells.

DISCUSSION

Cesarman et al. [6] examined 193 lymphoma tissues from HIV-infected and uninfected patients for the presence of HHV-8 DNA sequences by PCR amplification. Tissues from all eight patients with AIDS having a rare form of lymphoma, designated primary effusion lymphoma or body cavity-based lymphoma, contained HHV-8 DNA; all other lymphomas were negative for HHV-8 DNA. The hallmark of primary effusion lymphoma has been the presence of a malignant effusion in a body cavity (chest, abdomen, or pericardium) in the absence of a contiguous tumor mass [8,22]. All reported cases of primary effusion lymphoma have developed in adults, with the majority occurring in patients with AIDS [7,8]. Thirty-eight of 44 cases (86%) of primary effusion lymphoma examined for the presence of HHV-8 DNA by PCR have been positive [8,15,23–30]. In addition, five other non-effusion lymphomas have contained HHV-8 DNA [25,28,31].

This lymphoma, the first case of an HHV-8-associated lymphoma reported in a child and also the first HIV-infected female reported with an HHV-8-associated lymphoma, demonstrates several differences from the 43 HHV-8-associated cases reported in adults (Table I). The most notable difference is the small, non-cleaved cell morphology in this case contrasted with the large cell pleomorphic or immunoblastic morphology seen in HHV-8-associated lymphomas in adult patients. In the published literature to date, 99 samples of small non-cleaved cell lymphoma tissues (47 from HIV-infected patients and 52 from HIV-uninfected patients) have been examined, and none have contained HHV-8 DNA [6,8,24,28,30,32,33]. Albini et al. [25] recently reported three non-effusion lymphomas containing HHV-8 but did not state the cell type. Mass lesions were identified in this patient's abdomen and heart, although no tissue samples of these lesions were available for study. These masses likely represented other sites of lymphoma, consistent with the known pathology of small non-cleaved cell lymphoma.

The oligoclonal IgG rearrangement identified in this child's cells differs from the clonal rearrangements found

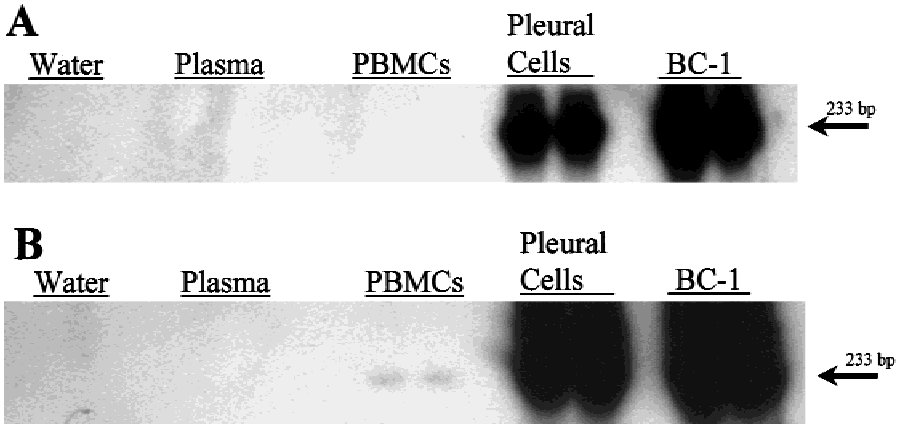

Fig. 5. Amplification of HHV-8 sequences from patient specimens. Duplicate samples of water (negative control), patient samples (plasma, PBMCs, pleural fluid cells), and BC-1 cells [42] (positive control) were amplified using HHV-8-specific primers by standard PCR (A) and nested PCR (B). The positive controls and pleural fluid cells yielded a positive signal (band at 233 bp) using both PCR methods. The PBMCs were positive only using nested PCR amplification. The water controls were all negative.

TABLE I. Comparison of Clinical and Laboratory Features of HHV-8-Associated Lymphomas in Adults to Present Pediatric Case*

Feature	Present pediatric case	Adult cases [8,15,23–28,30,31] (N = 43)[a]
HIV infected	Yes	36/43 (84%)
Gender		
Male	—	22
Female	Yes	3[b]
Not reported	—	18
Site of malignancy[c]		
Body cavity effusion	Yes	38
Other	Yes	9[d]
Tissue morphology		
Small non-cleaved cell lymphoma	Yes	0
Large cell lymphoma	—	32/32
Epstein-Barr virus		
Present in tumor	Yes	29/39 (74%)
Monoclonal	ND	19/19
Immunoglobulin rearrangement	Yes (Oligoclonal)	19/20 (95%) (Monoclonal)
c-*myc* rearrangement	Yes	0/20

*HHV-8, human herpesvirus-8; ND, not done. Note: This table lists only those lymphomas in adults that were tested and positive for HHV-8 DNA.
[a]Excludes one reference [44] due to incomplete histologic and clinical data.
[b]All 3 females were HIV-negative.
[c]Number of patients having malignancy at each specified site; some patients had malignancy at more than one site.
[d]Other sites included: soft tissue (1); soft tissue and bone marrow (1); soft tissue, pericardium, and myocardium (1); submandibular gland and lymph nodes (1); lung and lymph nodes (1); brain (1); and unspecified sites (3). Five patients without an effusion had HHV-8 identified in malignant tissue.

in >95% of adult patients with primary effusion lymphomas. Another contrasting feature of this case is the presence of a c-*myc* rearrangement and a t(8;14)(q24;q32) translocation in the pleural fluid cells, which occur commonly in small non-cleaved cell lymphomas. None of the HHV-8-associated lymphomas reported in adult AIDS patients have had c-*myc* rearrangements (Table I).

This patient had serum antibodies against lytic but not latent HHV-8 proteins. This is not unusual in patients with HHV-8-associated malignancies. Antibodies to latent HHV-8 proteins are undetectable by IFA in 12–29% of adult AIDS patients with KS [16] despite the detection of HHV-8 DNA in >95% of KS tissues [34]. Cross-reactivity of HHV-8 antibodies to EBV lytic antigens has been observed in some studies [13] but not others [35,36]. It is possible that antibodies to latent viral antigens are not detectable as a result of a poor immune response in this patient, the relative insensitivity of the tests used, or possibly as a result of HHV-8 strain differences.

The identification of HHV-8 in the genital tract of males [37] and females (C.T. Leach, unpublished data) suggests that this child's HHV-8 infection was acquired perinatally. This route of acquisition is suggested by previous studies reporting the identification of HHV-8 in blood and KS tissues from other children [38,39]. Unfortunately, blood from family members was not available for serologic testing. The epidemiology of HHV-8 in children, and in children born to HIV-infected women, is not known.

The strong epidemiologic association between HHV-8 and primary effusion lymphomas suggests a possible etiologic relationship. Although quantitative PCR was not performed on this patient's specimens, a much stronger signal was observed for pleural fluid cells compared with a comparable number of PBMCs (Fig. 5). It is possible that HHV-8 was present only in one or two of the three cell populations identified by karyotyping, or only in nonmalignant cells, and was not directly involved in the pathogenesis of this child's cancer. Because additional pleural effusion was not available for in situ hybridization, it was not possible to determine if only a subpopulation of cells contained HHV-8.

It has been postulated that HHV-8 is singularly responsible for malignant transformation in primary effusion lymphomas since the virus closely resembles two other transforming herpesviruses, EBV and herpesvirus saimiri. HHV-8 encodes analogues of cell growth regulators such as IL-6, cyclin D and G-protein-coupled re-

ceptors [14] that could promote cellular proliferation leading to malignant transformation. Growth stimulation of neoplastic cells may be accomplished in a paracrine manner, as suggested by recent studies of multiple myeloma [40]. Recently, Muralidhar et al. [41] demonstrated oncogenicity of a small latency-associated protein named kaposin, which is also produced in HHV-8 cell lines. Alternatively, HHV-8 may sometimes act in conjunction with EBV in causing malignancy. Most cases (74%) of HHV-8-associated lymphomas in adult patients also harbor EBV (Table I). Quantitation of EBV DNA in this child's malignancy, not studied for the previously-reported HHV-8-associated lymphomas, revealed approximately only 1 copy of EBV per 10 pleural fluid cells. This suggests separate HHV-8 and EBV infection of different populations of pleural fluid cells [15] or coinfection of a subpopulation of cells [42]. Either scenario may result in a milieu of cytokine production that fosters malignant transformation of B-cells. The c-*myc* rearrangement is characteristic of EBV-associated tumors in immunocompromised hosts [43]. The relationship between c-*myc* rearrangement and HHV-8 and EBV infection in these tumors remains to be ascertained.

In summary, we describe a case of an HHV-8-associated small non-cleaved cell lymphoma in a female child with AIDS. Malignant cells in this case differed from HHV-8-associated primary effusion lymphomas in adults with respect to cell morphology, oligoclonal immunoglobulin rearrangement, and c-*myc* rearrangement. Based on the above findings, this patient's lymphoma does not fit the criteria for primary effusion lymphoma proposed by Nador et al. [8]. These differences suggest that pathophysiologic mechanisms may differ among different HHV-8-associated malignancies. Further epidemiologic studies are needed to determine the spectrum of pediatric malignancies associated with this new human herpesvirus.

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